(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 28 August 2003 (28.08.2003)

PCT

(10) International Publication Number WO 03/070959 A3

(51) International Patent Classification7: C12N 9/02, 15/53 C12P 7/26,

(21) International Application Number: PCT/EP03/01537

(22) International Filing Date: 15 February 2003 (15.02.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

020 03 968.1

22 February 2002 (22.02.2002) EP

- (71) Applicant (for all designated States except US): ROCHE VITAMINS AG [CH/CH]; 124 Grenzacherstrasse, CH-4070 Basle (CH).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): SHIMIZU, Sakayu [JP/JP]; 6-9 Yamashita-cho, Tokiwa, Ukyo-ku, Kyoto-shi, Kyoto-fu 616-8212 (JP). WADA, Masaru [JP/JP]; E-204, 38-8-1 Kenjyojima, Matsuoka-cho, Yoshida-gun, Fukui-ken 910-1142 (JP).
- (74) Agent: LEDERER & KELLER; Prinzregentenstrasse 16, 80538 München (DE).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report:
 16 October 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PROCESS FOR PRODUCING LEVODIONE

(57) Abstract: An enone reductase characterized by a molecular mass of $61,300 \pm 5,000$ Da; NADPH and NADH as co-factor; a temperature optimum of $55-60^{\circ}$ C at pH 7.4; a pH optimum of 4.5-8.5 and a substrate specificity on α,β -unsaturated ketones, especially derived from a yeast and a process for the preparation of levodione from ketoisophorane.

Interior onal Application No

	INTERNATIONAL SEARCH REPOR	`'	PCT/EP 03/01537						
A. CLASSII	FICATION OF SUBJECT MATTER C12P7/26 C12N9/02 C12N15/	 53							
	01217720								
According to	According to international Patent Classification (IPC) or to both national classification and IPC								
B. FIELDS	SEARCHED								
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12P C12N									
Documentat	ion searched other than minimum documentation to the extent that	such documents are includ	ed in the fields searched						
	Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched								
	ata base consulted during the international search (name of data be								
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, FSTA, CHEM ABS Data									
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT								
Category °	Citation of document, with indication, where appropriate, of the re	levant passages	Relevant to claim No.						
X	VAZ ALFIN D N ET AL: "Old yello Aromatization of cyclic enones a mechanism of a novel dismutation	1-18							
	reaction." BIOCHEMISTRY, vol. 34, no. 13, 1995, pages 4246-4256,								
į	XP001164161 ISSN: 0006-2960 the whole document								
1		,	Ì						
}		-/							
ł									
X Furt	her documents are listed in the continuation of box C.	X Patent family n	nembers are listed in annex.						
° Special ca	ategories of cited documents:		shed after the International filing date not in conflict with the application but						
consid	ent defining the general state of the art which is not dered to be of particular relevance		the principle or theory underlying the						
filing		cannot be consider	ar ratevance; the claimed invention ed novel or cannot be considered to a step when the document is taken alone						
which citatio	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another cliention or other special reason (as specified) cannot be considered to involve an inventive step when the								
other	O' document referring to an oral disclosure, use, exhibition or other means document is combined with one or more other such document is combined with one or more other such documents, such combination being obvious to a person skilled								
latert	ent published prior to the international filing date but hen the priority date claimed	*&* document member of the same patent family							
Date of the actual completion of the international search Date of mailing of the international search report									
2	26 August 2003	11/09/20	11/09/2003						
Name and	mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2	Authorized officer							
NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016		Devijver, K							



International Application No PCT/EP 03/01537

		PC1/EP U3/U153/				
C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT						
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.				
X	WANNER PETER ET AL: "Purification and characterization of two enone reductases from Saccharomyces cerevisiae" EUROPEAN JOURNAL OF BIOCHEMISTRY, BERLIN, DE, vol. 255, no. 1, July 1998 (1998-07), pages 271-278, XP002202649 ISSN: 0014-2956 the whole document	1-18				
Ρ,Χ	KATAOKA M ET AL: "OLD YELLOW ENZYME FROM CANDIDA MACEDONIENSIS CATALYZES THE STEREOSPECIFIC REDUCTION OF THE C = C BOND OF KETOISOPHORONE" BIOSCIENCE, BIOTECHNOLOGY AND BIOCHEMISTRY, XX, XX, vol. 66, no. 12, December 2002 (2002-12), pages 2651-2657, XP009011699 ISSN: 0916-8451 the whole document	1-18				
P,X	WADA M ET AL: "PRODUCTION OF A DOUBLY CHIRAL COMPOUND, (4R,6R)-4-HYDROXY-2,2,6-TRIME THYLCYCLOHEXANONE, BY TWO-STEP ENZYMATIC ASYMMETRIC REDUCTION" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, WASHINGTON,DC, US, vol. 69, no. 2, February 2003 (2003-02), pages 933-937, XP009011700 ISSN: 0099-2240 the whole document	1–18				
A	EP 1 074 630 A (HOFFMANN LA ROCHE) 7 February 2001 (2001-02-07) the whole document	1–18				
A	US 4 072 715 A (BOGUTH WALTER ET AL) 7 February 1978 (1978-02-07) cited in the application the whole document	1-18				
A	STOTT KELVIN ET AL: "Old Yellow Enzyme: The discovery of multiple isozymes and a family of related proteins." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 9, 1993, pages 6097-6106, XP002252405 ISSN: 0021-9258 the whole document	1-18				



INTERNATIONAL SEARCH REPORT

Interconal Application No PCT/EP 03/01537

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT Category of Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.	
Category °	Citation of document, with indication, where appropriate, of the relevant passages NIINO YUKO S ET AL: "A new Old Yellow Enzyme of Saccharomyces cerevisiae." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 5, 1995, pages 1983–1991, XP002252406 ISSN: 0021–9258 the whole document	Relevant to claim No.	





Interior on al Application No PCT/EP 03/01537

		,		
Patent document cited in search report	Publication date	Patent memb		Publication date
EP 1074630 A	07-02-2001	BR 000 CA 231 CN 128 JP 200106	74630 A2 03302 A 15837 A1 05410 A 51488 A 28991 B1	07-02-2001 05-06-2001 02-02-2001 28-02-2001 13-03-2001 06-08-2002
US 4072715 A	07-02-1978	US 398 US 409 US 419 AT 34 AT 64 BE 83 DE 253 FR 236 FR 236 FR 236 GB 156 GB 156 JP 5500	05533 A5 05533 A5 05038 A 05038 A 056100 A 047422 B 045575 A 032565 A1 03797 A1 03798 A1 03798 A1 03798 A1 08195 A 08195 A 01692 B 09256 C 07277 B 09925 A ,B, 06949 A 08196 A	29-09-1978 26-10-1976 13-06-1978 22-05-1979 27-12-1978 15-05-1978 20-02-1976 04-03-1976 08-10-1976 08-10-1976 08-10-1976 19-04-1978 19-04-1978 19-04-1978 10-01-1980 19-09-1984 08-03-1980 13-01-1984 29-05-1984 20-07-1976 09-02-1983 24-02-1976 31-05-1977 19-04-1978